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Clinical, Epidemiologic, and Virologic Studies in Four Clusters of the Chronic Fatigue Syndrome

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• ***Background.*—The purpose of this study is to provide a case definition of chronic fatigue syndrome in an outbreak occurring in the Nevada-California region to evaluate candidate etiologic agents and observe the natural history of the illness.**

***Methods.*—Patients diagnosed as having chronic fatigue syndrome were studied by repeated interviews, questionnaires, and blood collection over a 3-year period. Serum samples were tested for antibodies to Epstein-Barr virus, human herpesvirus-6, and human T-lymphotropic viruses I and II. Leukocytes from typical cases were also assayed for human T-lymphotropic viruses I and II.**

***Results.*—Cases were defined as persons who had: (1) severe persistent fatigue following an acute illness appearing in an individual with no previous physical or psychological symptoms; (2) presenting signs and symptoms of an acute infection; (3) severe and persistent headache and/or myalgias; and (4) abrupt change in cognitive function or the appearance of a new mood disorder. After 3 years of follow-up, almost all study subjects were able to return to pre-illness activity. None of the viruses evaluated—human T-lymphotropic viruses I and II, Epstein-Barr virus, or human herpesvirus-6—could be etiologically linked to these outbreaks.**

***Conclusion.*—Clinical features of outbreaks of chronic fatigue syndrome differ sufficiently to suggest different etiologic agents. Giardiasis appears to have precipitated one of the four clusters in this study but the cause(s) of the other three outbreaks is as yet uncertain. The overall prognosis of chronic fatigue syndrome is usually favorable.**

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A syndrome of chronic fatigue, frequently accompanied by elevated antibodies to the Epstein-Barr virus (EBV), has been noted in recent years.[1-3] There has been considerable controversy as to whether this disease is synonymous with chronic EBV infection; indeed, there is some disagreement as to whether the chronic EBV syndrome is an entity.[4] Progress in the evaluation of the chronic fatigue syndrome (CFS), chronic EBV, and related illnesses has been hampered by variable clinical and laboratory criteria used for disease definition.[4-7] In 1985 (*The Sacramento Bee.* October 11, 1985:B), the first of several articles described an epidemic of CFS in the Lake Tahoe (Nevada and California) area.[8,9] This outbreak was associated with neurologic symptoms, raising comparisons with early reports of clusters referred to primarily as Iceland disease, benign myalgic encephalomyelitis, the post-viral fatigue syndrome, or epidemic neuromyasthenia.[10-20] Noting the confusion in this field that has resulted in part by the inclusion of a wide range of overlapping clinical conditions, we have investigated four reported outbreaks of CFS with two objectives: first, to develop a case definition that would illustrate the primary manifestations of this disease in the Nevada-California cluster, and second, to evaluate the potential roles of EBV, human herpesvirus 6 (HHV-6), and human T-lymphotropic viruses I and II (HTLV-I and -II) in this syndrome as we have defined it.

In late 1984, two of us (P.C. and D.P.) practicing internal medicine in Incline Village, Nev, situated on the northeast shore of Lake Tahoe, became aware of a sudden increase in the number of patients complaining of chronic fatigue and abnormalities in cognitive function. This outbreak, which peaked in the summer of 1985, has been described in part elsewhere.[6-9]

Coincidentally, a somewhat similar illness affected students and staff of a high school in Truckee, Calif, located 40 miles northwest of Lake Tahoe. During the fall of 1984, a number of cases of infectious mononucleosis had occurred at this school. The incidence was not considered unusual, but two students suffered a prolonged course of fatigue and mononucleosislike symptoms, and subsequently faculty members began to be affected in February 1985. Within 6 months, approximately one third of the high school faculty reported symptoms of chronic fatigue. All of the affected faculty members shared the same study room.

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In December 1985, approximately 5 months after the peak of the outbreak in the Lake Tahoe region, a family practice physician in Yerington, Nev, a rural community 73 miles east of Incline Village, noted an outbreak of chronic fatigue in her community in association with a series of positive Monospot test results and atypical lymphocytosis.

The fourth outbreak began in Placerville, Calif, 95 miles west of Incline Village, early in 1986 and was associated with an epidemic of giardiasis. The giardiasis epidemic appeared to coincide with a period of drought and increased turbidity of the water supply used by the community outside Placerville.

METHODS

Questionnaires and Blood Collection

An in-person interview questionnaire concerning clinical history and symptoms was developed prior to a visit to these communities in September 1986, 1 year after the first report of the outbreak (*The Sacramento Bee*. October 11, 1985:B1) and was used for all individuals participating in the study. This questionnaire consisted of an introduction obtaining informed consent, identifying data (including age, race, sex, occupation, prior education, and residential history) and a series of questions regarding specific signs and symptoms (including fever, fatigue, weight loss, unexplained rashes, diarrhea, conjunctivitis, cough, sore throat, mood changes, cognitive disorders, and other neurologic abnormalities) that were present at the onset of illness and at the time of interview (October 1986). In contrast to the initial study of this cluster,[9] where 134 patients were interviewed briefly by telephone, we chose to use in-depth interviews to catalog the most typical cases in this particular outbreak. Of the approximately 80 patients in the Incline Village and Truckee communities evaluated by the two physicians reporting the cluster, 11 were chosen by these physicians as being representative of their patient group on the basis of acute onset of a new debilitating prolonged illness in a patient they had known to have no preexisting physical or psychologic health conditions. The 11 patients in Yerington consisted of all affected individuals identified by the community physician who could attend the Yerington clinic on the day of our visit. In Placerville, where the outbreak was reported by a local newspaper editor and had not been systematically evaluated by a physician, nine participants were selected by this editor who had coordinated an effort to investigate the concomitant outbreak of giardiasis and was familiar with those most severely affected. All participating individuals signed an informed consent, were interviewed with the questionnaire, and blood samples were collected for serologic studies. Serum samples were separated from the blood samples on the day of phlebotomy and were sent to the National Cancer Institute Repository, Frederick, Md, for immediate aliquoting and freezing. All samples were stored at −70°C until the time of testing.

Three groups of controls were used for serologic evaluation. In Incline Village, blood samples were collected from nine healthy donors or patients with no evidence CFS who were seen at the same time as the patients with the CFS and were in the same age range (contemporary control subjects). Twenty-five frozen serum samples, which had been drawn during the previous 3 weeks from adults with identified illnesses who did not have symptoms of CFS, were obtained from the hospital laboratory in Yerington (community control subjects). A third control group consisted of 70 serum samples that were selected from a collection of serum samples previously obtained from healthy volunteer blood donors in Burlington, Vt, in 1981; the 70 serum samples were selected from donors in the same age range as the patients with CFS (blood donor control subjects).

Ten months after the first questionnaire was given, a questionnaire was mailed to all study subjects to initiate a 1-year follow-up on the course of illness. Nonrespondents were contacted by telephone. A second follow-up telephone survey was performed 23 months after the visit to the communities. Twenty-eight of the original 31 patients were evaluated in this second follow-up; the other three were not available for interview. Patients were questioned in detail about the course of their disease, including time of maximum severity, and their degree of recovery involving evaluation of 10 specific parameters. Five of these were physical abilities (personal care, walking, working, sleeping, and coordination) and five were mental functions (concentration, speech, memory, calculations, and orientation). For each parameter, the degree of function was rated on a scale of 0 to 2, with a 0 indicating severe impairment. In addition, each patient was asked the percentage of function at the time of interview compared with the preillness state.

In June 1989, 44 months after the first questionnaire, a final questionnaire to determine subsequent health problems and performance status was mailed to the 31 patients. Of these 31, one had died of unrelated causes and responses were obtained from 29 of the other 30.

Virologic Assays

Antibody to HHV-6 was measured by an immunofluorescence assay as previously described[21] using the chronically infected HSB-2 cell line as the source of HHV-6 antigen. Antibodies to the EBV viral capsid and early antigens were measured by immunofluorescence as previously described.[22,23] The following enzyme-linked immunosorbent assay kits were used to assay serum samples for antibodies to HTLV-I and -II: HTLV-I whole virus enzyme-linked immunosorbent assay (NEA-510A, E. I. DuPont Nemours and Co, Wilmington, Del), HTLV-I enzyme-linked immunosorbent assay (6017, Cambridge Bioscience Recombinant, Worcester, Mass), whole virus SynthEIA HTLV-I enzyme immunoassay (EIA) (Olympus Corp, Lake Success, NY), peptide SynthEIA HTLV-I(s) EIA (Olympus, RV900250), and peptide SynthEIA HTLV-II EIA (Olympus, RV900750). Serum samples were diluted 1:20 in each kit's sample diluent prior to testing. Samples were scored positive or negative in each assay according to the manufacturer's cutoff described in each kit protocol.

In addition, serum samples from nine donors with "classic" (4+, see below) CFS were tested by Western blot[24] using HTLV-I and -II antigens despite being screen negative by the enzyme-linked immunosorbent assay. These same nine donors were also tested by polymerase chain reaction using freshly frozen viable leukocytes. Polymerase chain reaction was performed as previously described[25] by lysing 1×10^6 peripheral blood lymphocytes in 0.5 mL of a buffer containing 0.001% Triton X100 and 0.0001% SDS plus proteinase K at a concentration of 30 μg/mL for 1 hour at 56°C. Samples were then heated at 95°C for 15 minutes and frozen until used. Primers to a generic sequence of the HTLV-I and -II pool region were used (SK110 and SK111) to amplify sample DNA and probes specific for HTLV-I (SK112) or -II (SK188) were used to determine HTLV specificity.[25] Controls consisted of peripheral blood lymphocytes from HTLV-I and -II–infected individuals and positive polymerase chain reaction signals were obtained using the appropriate primers and probes. Normal individual control subjects not infected with HTLV-I and -II or samples containing no DNA were negative when amplified with these primers and probes.

Statistical Methods

Differences in geometric mean titers of antibody between groups were tested for statistical significance using the standard t test. Differences between groups in proportions were tested using standard χ^2 tests. For both, $\alpha=.05$ was the significance level.

Case Definition

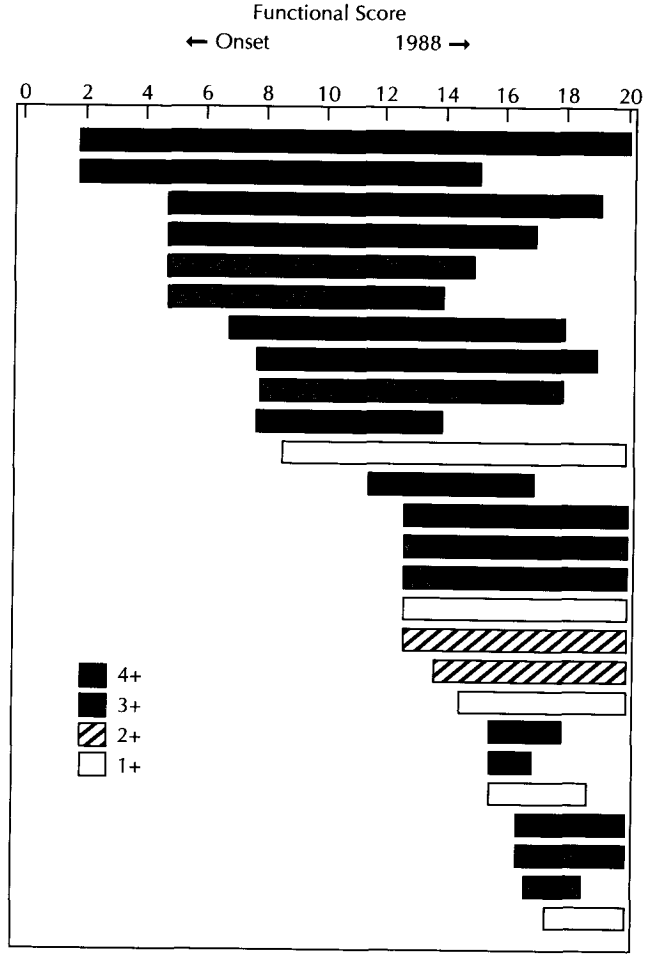
After all participants were interviewed but prior to the testing of viral antibodies, a clinical case definition was developed based on the most consistent and striking features described by those with the most severe symptoms subsequent to an apparent acute infectious illness. These were (1) severe persistent fatigue following an acute illness appearing in an individual with no previous physical or psychological symptoms; (2) presenting signs and symptoms of an acute infection including sore throat and/or

Table 1.—Disease Patterns in Four Groups of Patients With Chronic Fatigue Syndrome

	Incline Village, Nev/ Truckee, Calif*	Yerington, Nev	Placerville, Calif
Mean age,y (range)	40.7 (12-55)	31.1 (14-46)	41.1 (26-65)
Sex, M/F	4/7	3/8	4/5
Acute onset, No. (%)	10/11 (90)	8/11 (73)	3/9 (33)
Sore throat	10/11 (90)	6/11 (54)	2/9 (22)
Lymphadenopathy	10/11 (90)	7/11 (64)	7/9 (79)
Pain, No. (%)	8/11 (70)	8/11 (73)	6/9 (67)
Headache	6/10† (60)	6/11 (54)	5/9 (55)
Myalgias	4/10† (40)	4/11 (36)	4/9 (44)
Central nervous system disorders, No. (%)	8/10† (80)	9/11 (82)	8/9 (89)
Cognitive disorder	8/10† (80)	2/11 (18)	6/9 (67)
Mood changes	8/10† (80)	9/11 (82)	7/9 (78)
Final classification, No. (%)			
4+	6/11 (54)	2/11 (18)	3/9 (33)
3+	3/11 (27)	3/11 (27)	3/9 (33)
2+	2/11 (18)	4/11 (36)	3/9 (33)
1+	. . .	2/11 (18)	. . .

*Incline Village and Truckee are combined because of simultaneous onset, proximity, and care of Truckee patients by physicians in Incline Village.

†No information on one patient.



Clinical course of epidemic chronic fatigue syndrome. Functional scores of 26 study subjects at the onset of illness and the time of 1988 follow-up.

lymphadenopathy; (3) severe and persistent headache and/or myalgias; and (4) abrupt change in cognitive function or the appearance of a new mood disorder.

Individuals with all four features were classified as being typical (4+) cases for the purpose of clinical-serologic correlation. Persons with three of the four features were classified as 3+, two features as 2+, and one feature as 1+.

RESULTS

Evaluation of Clusters and Follow-up

Variability in presenting signs and symptoms were observed in each of the four clusters (Table 1, Incline Village and Truckee are combined because of simultaneous onset, proximity, and care of Truckee patients by Incline Village physicians). Within each cluster there were patients who had the characteristic features described in our case definition but there were also individuals whose onset was of long duration and not associated with an acute infectious syndrome. Patients in Placerville frequently complained of diarrhea and paresthesias, features that were virtually absent in the other outbreaks. Another distinguishing feature appeared to be in the pattern of infection, patients in Truckee, Incline Village, and Yerington citing illness among family members and other close contacts more frequently than those in Placerville[6] (see the Comment section).

After 2 years of follow-up, 12 of the 26 patients who could be evaluated were functioning without any limitations, including three of the 10 available classic (4+) cases (Fig 1). After 3 years of follow-up, almost all study subjects were able to return to pre-illness activity. Of the 11 most typical (4+) patients, eight reported a return to at least 75% of their pre-illness energy level, but normal activity was often possible only at a somewhat decreased level.

Clinical/Laboratory Correlation

Serologic evaluation for HHV-6 antibody was possible for only 27 of 31 patients and 89 of 105 control subjects because some samples were exhausted in initial HHV-6 studies using infected cord blood, an assay subsequently shown to be insensitive and less reproducible. All 116 serum samples were positive for HHV-6 antibody with the exception of two control subjects. As shown in Table 2, the geometric mean titers for cases (1:132.6) was higher than for control subjects (1:87.9) but the difference was not statistically significant (t=1.23). Similarly, high titers (≥1:320) were found less frequently in control subjects (25/89) or less typical (1 to 3+) cases (5/18) than typical (4+) cases (5/9, odds ratio=3.2) but the trend was not statistically significant (P=.22).

Antibody titers to EBV early antigen (but not viral capsid antigen) were higher in patients than in control subjects, but no significant differences between typical cases (4+) and less typical cases (1 to 3+) were found (Table 2). On direct comparison of serum samples, little correlation was found between HHV-6 and EBV antibodies in

Table 2.—GMT of Antibody HHV-6 and EBV in Study Groups*

Study Group	No., All Cases	Tested for Antibodies†					
		HHV-6		EBV-VCA		EBV-EA	
		No.	GMT	No.	GMT	No.	GMT
Cases (4+)	11	9	186.6	8	207.5	8	7.5
Cases (1-3+)	20	18	111.7	16	257.7	16	5.4
Total	**31**	**27**	132.6	**24**	239.7	**24**	6.0
Control subjects	105	89	87.9	49	254.0	49	2.1

*GMT indicates geometric mean titers; HHV-6, human herpesvirus-6; EBV, Epstein-Barr virus; VCA, viral capsid antigen; and EA, early antigen. Note, titers of less than 1:10 included in calculations of GMTs (=1).

†Titers were not obtained in all patients and control subjects due to insufficient serum.

patients with CFS or control subjects. No significant correlation was found between antibody titers and clinical course of illness.

Studies directed at detection of HTLV-I and -II found no antibodies by Western blot and no evidence of HTLV-I and -II pol genomic sequences of polymerase chain reaction.

COMMENT

Epidemics of what today might be called CFS have been reported for more than 50 years,[9-19] but recently have gained increased attention from the scientific and lay community because of recent developments in the laboratory[5,7,26,27] and considerable attention in the news media[8] (*The Sacramento Bee.* October 11, 1985:B1. *Newsweek.* November 12, 1990:62-70). The development of a number of support groups has been of considerable assistance to individuals complaining of chronic fatigue. However, the diversity of signs and symptoms and the likelihood of multiple causes included in CFS make it important to improve the criteria for subclassification of patients to facilitate specific therapeutic trials and to improve prognostic capabilities. In an attempt to develop criteria for the evaluation of CFS several investigators recently reported a working case definition.[28] Because of the particular opportunities afforded by outbreaks to identify subcategories of illness included in the broad spectrum of illnesses affecting patients who might be diagnosed using this case definition, we describe our experience with these four clusters with particular attention to an identifiable subset meriting further etiologic studies.

In this study, we interviewed and obtained blood samples from four clusters of patients who complained of CFS with a variety of associated signs and symptoms. As part of this investigation, it was apparent to us that publicity involving this epidemic as well as concomitant activities of a National Chronic EBV Society were bringing individuals into this cluster with widely diverse symptoms probably etiologically unrelated. Because of the frequent absence of objective findings as well as the prominence of psychological manifestations, we therefore developed a case definition that required an abrupt, well-documented, persistent infectious disease syndrome occurring in a previously healthy individual with no evidence of psychological or physical disorders. We did not specify a minimum period of symptoms (the working definition of CFS including 6 months of symptoms[28] had not yet been published) but all of our 3+ and 4+ patients had been ill for more than 6 months. Severe pain and disturbances in cognitive function were important parameters not only because of their severity but also their susceptibility to objective study.

This study differs from other investigations of the Incline Village cluster[8,9] in several ways. The additional communities studied created a more economically diverse study group. In addition, the intensive interview led to subclassification of reported cases. Finally, long-term follow-up of the individuals involved allowed characterization of the course of disease. We did not attempt to sample the entire cluster, but rather to identify a sizeable group of "typical" (4+) cases, as assessed by the attending physicians seeing patients during these particular outbreaks.

This study illustrates the importance of careful interview in attempting to distinguish CFS from other illnesses. The correlation between serology and case classification supports this distinction, even though the viral patterns may be a result rather than a cause of the disease process. Patients with typical postinfectious CFS showed an abrupt change in life-style caused by this illness. Among the patients we observed were a marathon runner, a marathon bicyclist, avid skiers, mountain climbers, and other individuals pursuing an unusually physically active life-style prior to their acute illness. All of these patients attempted to continue their activities and most of them, after periods ranging from 6 to 18 months, eventually were able to resume their previous activities.

It should be noted that there are possibly important differences between these patients and many of those affected in other outbreaks. The group studied by Dillon et al[19] reported as epidemic neuromyasthenia, for example, was notable for an absence of changes in cognitive functions. In 1957, Skelokov et al[17] reported epidemic neuromyasthenia in student nurses and their group also had several differences from those in our study; cognitive disorder was not a prominent feature and the local muscle weakness differed both from our cases and from those of White and Burtch,[12] who reviewed a cluster of Iceland disease in New York. Their group had muscular tenderness and dysesthesia as prominent features and the headaches were mainly occipital; few had lymphadenopathy and no disturbances of cognitive function were reported, although depression was a common feature in this series as well as of the others. In contrast to the above clusters, one outbreak that did have cases reminiscent of those that we observed was evaluated by Poskanzer et al[16] in Punta Gorda, Fla, and by Roueche[29] who found severe cognitive disorder but few other objective neurologic signs.

Even in our four clusters, epidemiologic and clinical features appeared to distinguish Placerville from the other three clusters. In addition to the more prominent gastrointestinal symptoms and paresthesia, the Placerville outbreak had the appearance of being more community-wide than the other clusters, particularly the Truckee cluster that appeared to focus around the community high school. Distinguishing between case-to-case transmission vs a common environmental source is frequently difficult, but the teachers involved in the Truckee High School noted that all documented cases shared the same closed environment on an almost daily basis and they trace their common infection to one individual who allegedly returned to school while still symptomatic from his illness. The familial nature of the illness in Yerington and Truckee, described elsewhere,[6] was not as clearly documented in

Incline Village or Placerville but is also important because it demonstrated that the inciting agent could produce a variety of manifestations and that CFS may be the most extreme manifestation of an agent that can produce transient signs and symptoms in members of the same family as well as in the community.

In addition to careful epidemiologic description of individual clusters, characterization of disease manifestations and potential etiologic agents requires uniform and specific diagnostic evaluation. Patients reporting severe cognitive dysfunction should be sought for evaluation by more sophisticated neuropsychiatric evaluation, perhaps including magnetic resonance imaging and positron emission tomographic scans. Those with myalgias and muscle weakness should be studied by a battery of sophisticated assays on muscle biopsy specimens, such as those reported by Archard et al.[30] The series of articles[31-34] on brucellosis and influenza that suggest a psychological cause for postinfectious chronic fatigue are informative. It is apparent, however, that more standardized psychologic and neurologic evaluation of patients with this syndrome is needed, and the selection of specific groups for detailed study should allow the better characterization of patients for diagnostic and therapeutic trials.[5,35,36]

The herpesvirus HHV-6 antibody (HHV-6 and EBV) studies performed on our patients and control subjects, while indicating a difference between typical patients and control subjects, did not identify an etiologic agent and supported the finding of elevated antibodies to EBV and other viruses observed in an earlier investigation of one of the clusters[9] in this study. The isolation of HHV-6 from patients with lymphoproliferative diseases led us to attempt to determine if there was a relationship between disease status and HHV-6antibodies. The EBV serologic test was repeated in an attempt to assess the specificity of the HHV-6 antibody titers and as an indirect measurement of cellular immunity. While there was a suggestively higher frequency of antibodies to HHV-6 in patients with the epidemic CFS meeting all the criteria of our "typical" case definition compared with atypical cases and controls, studies indicating a high prevalence of this virus[37,38] and its appearance in the pediatric population[39] suggest that HHV-6, like EBV, may be a ubiquitous virus that is reactivated in outbreaks but not necessarily a causative factor. Two difficulties in this study that are being resolved by studies in progress are improved assay standardization and selection of controls (to include more contemporary age-and sex-matched community control subjects). The retroviruses HTLV-I and -II played no role in these cases; no evidence of antibody was detected by enzyme-linked immunosorbent assay or Western blot and no evidence of either virus was found by polymerase chain reaction. The absence of detectable retrovirus infection, in contrast to reports by DeFreitas et al,[26] may be due to differing associated agents in geographically different areas or to differences in assays and reagents used. Since the tests we used have been successful in identifying, isolating, and characterizing both HTLV-I and -II in a number of populations[24,40-45]; however, it is highly unlikely that either of these viruses played a role in the four communities we have investigated. In summary, the clusters in Truckee, Incline Village, and Yerington (but not Placerville) appeared to represent an outbreak in which a case definition resembled those reported in at least one other outbreak,[16,29] but did not resemble most descriptions of epidemic neuromyasthenia, benign myalgic encephalitis, or Iceland disease. While a constellation of other symptoms, including ataxia, joint pain, anorexia, and disturbance in sleep patterns were observed in some of our patients with typical disease, the inclusion of these symptoms in case definitions for describing other clusters is likely to identify many patients with unrelated disorders. Our findings provide specific clinical, epidemiologic, and laboratory documentations of the heterogeneity of CFS, emphasized in recent symposia that also noted the need to separate the evaluation of clusters from those of sporadic cases.[5,6] The difference in presentation between most patients in Placerville and those in the other clusters suggests different causes for these outbreaks (perhaps giardiasis in Placerville) and supports our contention that episodes of "epidemic neuromyasthenia" can be subdivided with careful attention to medical history, physical findings, and appropriate laboratory studies. The long-term prognosis for patients affected in outbreaks of CFS appears to be excellent.

Subsequent to the submission of this article, a study was published[46] describing clinical and laboratory studies in patients involved in two of the four clusters were investigated. Techniques used to search for neurologic involvement, immunologic abnormalities, and HHV-6 activation represent an example of the clinical and laboratory efforts that should be associated with detailed epidemiologic evaluation.

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